SUPPLEMENTAL MATERIAL

Neonatal Organophosphorus Pesticide Exposure Alters the Developmental Trajectory of Cell Signaling Cascades Controlling Metabolism: Differential Effects of Diazinon and Parathion

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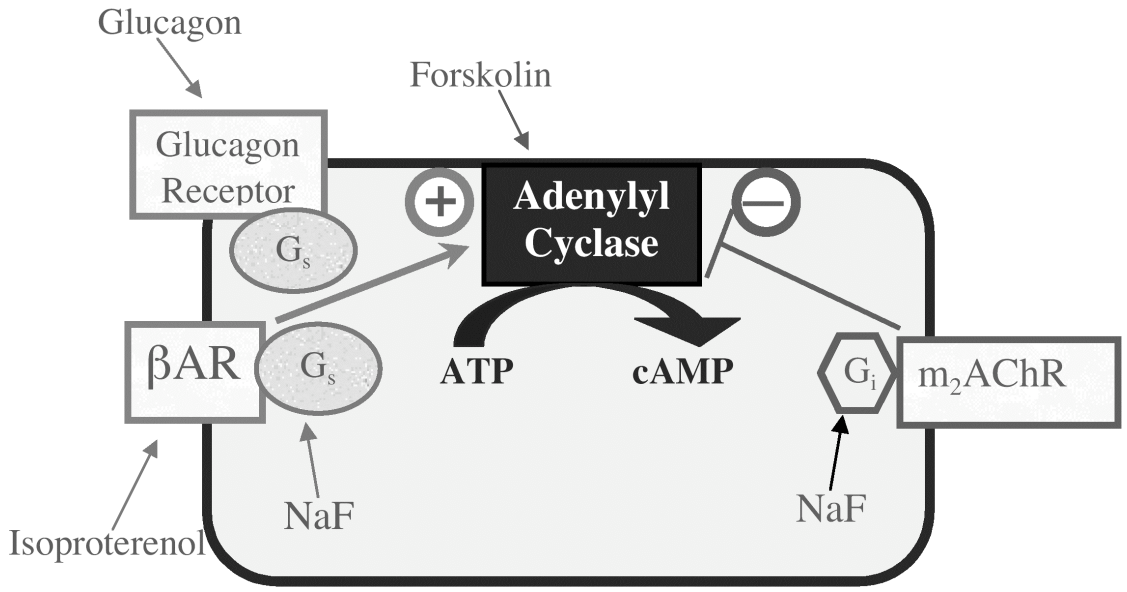
Supplemental Material, Figure 1

Mechanisms controlling AC activity, showing probes for each step in the pathway: isoproterenol for the βAR, glucagon for the glucagon receptor, NaF for the G-proteins, and forskolin for AC itself. Both βARs and glucagon receptors enhance AC activity through the stimulatory G-protein, G_s, whereas m_2AChRs inhibit AC through mediation of the inhibitory protein, G_i.



Supplemental Material, Table 1

Adenylyl Cyclase Activities and Receptor Binding in Controls

	PN30		PN60		PN100	
	Male	Female	Male	Female	Male	Female
Liver						
Basal AC[a]	3.1 ± 0.1	3.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.2	3.2 ± 0.1	3.0 ± 0.1
Isoproterenol-Stimulated AC[a]	4.8 ± 0.2	5.8 ± 0.2*	5.8 ± 0.1	6.3 ± 0.3	4.5 ± 0.2	4.6 ± 0.1
Glucagon-Stimulated AC[a]	29 ± 1	28 ± 1	34 ± 1	31 ± 1*	30 ± 1	27 ± 1*
NaF-Stimulated AC[a]	16.9 ± 0.5	16.9 ± 0.6	22.0 ± 1.1	21.5 ± 0.7	19.3 ± 0.4	18.8 ± 0.5
Forskolin-Stimulated AC[a]	58 ± 4	68 ± 2	77 ± 3	69 ± 2	73 ± 3	67 ± 3
βAR Binding[b]	3.2 ± 0.1	3.5 ± 0.2	3.6 ± 0.2	4.3 ± 0.2	2.7 ± 0.1	3.2 ± 0.1*
Heart						
Basal AC[a]	46 ± 1	44 ± 2	28 ± 1	29 ± 1	20 ± 1	23 ± 1*
Isoproterenol-Stimulated AC[a]	95 ± 2	95 ± 3	62 ± 2	67 ± 3	46 ± 2	54 ± 2*
Glucagon-Stimulated AC[a]	68 ± 2	68 ± 3	50 ± 2	50 ± 2	34 ± 2	40 ± 1*
NaF-Stimulated AC[a]	129 ± 3	127 ± 5	89 ± 2	93 ± 4	78 ± 3	83 ± 3
Forskolin-Stimulated AC[a]	803 ± 7	772 ± 22	646 ± 17	693 ± 24	554 ± 17	585 ± 16
βAR Binding[b]	11.1 ± 0.3	10.3 ± 0.3	8.0 ± 0.2	6.9 ± 0.3*	7.6 ± 0.4	7.3 ± 0.2
m_2AChR Binding[b]	183 ± 4	182 ± 6	161 ± 7	152 ± 7	170 ± 6	188 ± 6*
Cerebellum						
Basal AC[a]	—	—	—	—	166 ± 5	174 ± 4
Isoproterenol-Stimulated AC[a]	—	—	—	—	220 ± 7	208 ± 5
NaF-Stimulated AC[a]	—	—	—	—	212 ± 5	194 ± 5*
Forskolin-Stimulated AC[a]	—	—	—	—	1138 ± 45	1202 ± 60
βAR Binding[b]	—	—	—	—	22.5 ± 0.4	22.4 ± 0.4

Values are mean ± SE pooled across both sets of control cohorts (n=12 per sex at each age).
[a]pmol / min / mg protein; [b]fmol / mg protein; *significant difference between males and females

In control rats, liver and heart AC activities both showed robust responses to stimulants ($p<0.0001$ for the main effect of each stimulant compared to basal activity) but the relative response of each stimulant differed among tissues and ages, and between sexes: tissue × stimulant, $p<0.0001$; sex × stimulant, $p<0.0005$; age × stimulant, $p<0.0001$; tissue × age × stimulant, $p<0.0001$; age × sex × stimulant, $p<0.06$; tissue × age × sex × measure, $p<0.007$. Superimposed on the differences in response to stimulants, the overall temporal pattern of AC activity differed between liver and heart (tissue × age, $p<0.0001$; tissue × age × sex, $p<0.005$). As shown earlier (Navarro et al. 1991), liver AC declines sharply in the immediate postnatal period, whereas heart AC peaks in early adolescence and then declines; accordingly, here we saw an overall decrease in heart AC from adolescence to adulthood ($p<0.0001$ for the main effect of age), whereas liver AC showed a slight rise between PN30 and PN60 and a subsequent minor decline by PN100 ($p<0.0001$ for the main effect of age). Both tissues showed age- and sex-related differences in AC activity and/or stimulant responses, necessitating a point-by-point comparison of sex differences for each measure: liver, $p<0.06$ for age × sex, $p<0.003$ for sex × stimulant, $p<0.0001$ for age × stimulant, $p<0.007$ for age × sex × stimulant; heart, $p<0.08$ for age × sex, $p<0.0001$ for age × stimulant. Nevertheless, the individual sex differences were only sporadic, with the exception of the heart on PN100, where females showed significantly higher AC values than males (main effect of sex, $p<0.03$). The major response differences between liver and heart reflected disparities in the relative effects of the various AC stimulants. In the liver, glucagon produced a much larger response than did isoproterenol, whereas the opposite was true for the heart; this reflects the relatively greater physiologic importance of glucagon signals in the liver as compared to βAR signals in the heart. Similarly, in the liver, glucagon produced a greater stimulatory response than did fluoride, reflecting the mixed involvement of

both stimulation (G_s-related) and inhibition (G_i-related) for the latter agent; in the heart, isoproterenol produced a smaller signal than did fluoride. Finally, in the cerebellum, we again saw robust stimulatory responses to isoproterenol, fluoride and forskolin (all at $p<0.0001$) but without any sex differences (no main effect of sex or sex × stimulant interaction).

Because there were two control cohorts (controls for the DZN study, controls for the PRT study, each comprising 6 males and 6 females for each age point), the values were normalized and presented as a single set. However, statistical comparisons of the effects of DZN and PRT were made only with the appropriately matched control cohort.

REFERENCE

Navarro HA, Kudlacz EM, Slotkin TA. 1991. Control of adenylate cyclase activity in developing rat heart and liver: effects of prenatal exposure to terbutaline or dexamethasone. Biol Neonate. 60:127-136.